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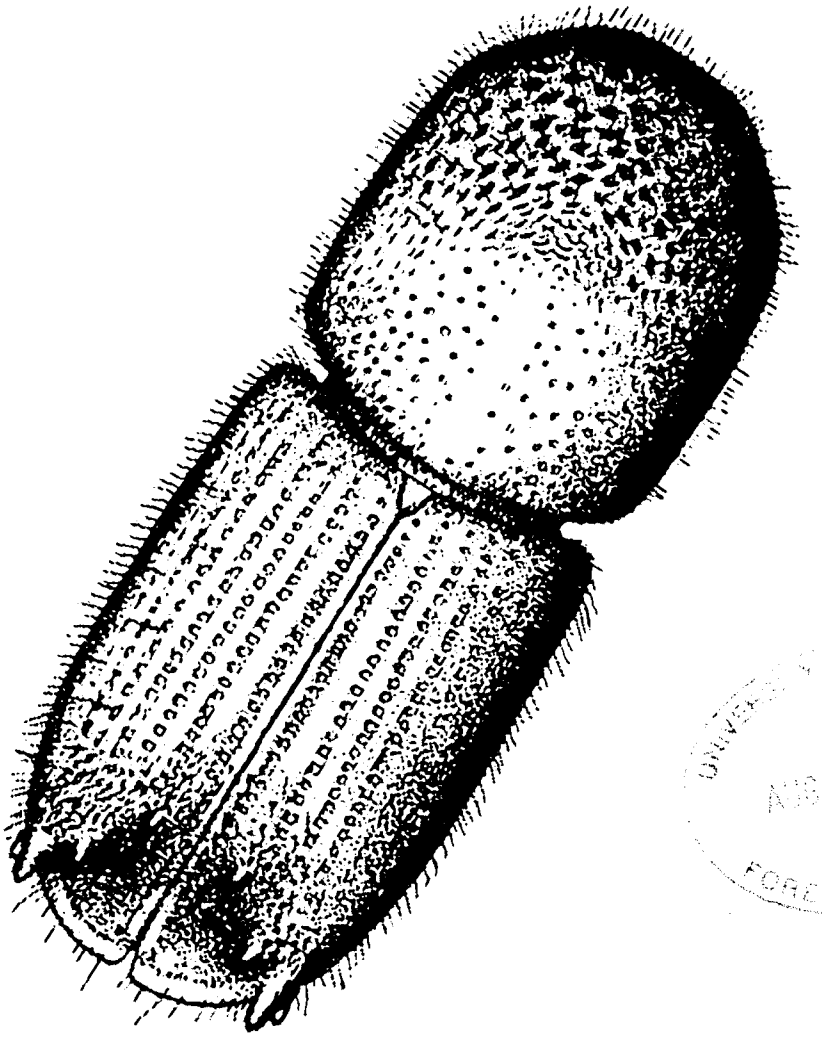
# Forest Health Protection Report

# Pheromone Attractant Compostion for the Pine Engraver Beetle: Southeast Alaska Population



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# Pheromone Attractant Composition for the Pine Engraver Beetle: Southeast Alaska Population

Special Report: R10-TP-117
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## Introduction

The pine engraver, *Ips pini* (Say), is a pest of pines throughout transcontinental North America (Furniss and Carolin, 1977; USDA, 1985). It colonizes the phloem of branches and stem material broken from standing trees during natural disturbances or logging operations (Furniss and Johnson, 2002). Under conditions of drought or high population density it can also colonize and kill healthy, standing trees or treetops. Southeast Alaska is near the northwestern extent of the range of *I. pini*. In this region of North America, the insect is associated with shore pine, *Pinus contorta* Dougl. var. *contorta*. Specimens of *I. pini* collected in 1954 and 1955 in *P. c. contorta* near Petersburg and Ketchikan, Alaska by W.F. McCambridge and in 1959 by D.E. Bright are deposited in the forest insect collection of the Pacific Northwest Research Station housed in Juneau, Alaska. Little is known of the biology of *I. pini* (or other associated scolytids) in *P. c. contorta* in southeastern Alaska.

The aggregation pheromone of *I. pini* is known to vary geographically across North America. Much of the variability can be explained by the effect of the enantiomeric composition (stereochemistry) of ipsdienol and the presence or absence of lanierone on the response of the beetles (Teale et al., 1991; Seybold et al., 1992, 1995). These differences in pheromone composition are also reflected in the genetic composition of the populations as assessed by analysis of mitochondrial DNA (Cognato et al., 1999). This study was designed to evaluate: (1) the bark beetle complex associated with *P. c. contorta*; (2) the combination of semiochemicals (pheromones) that are attractive to *I. pini* in the shore pine type; and (3) the semiochemicals that are produced by *I. pini* in *P. c. contorta*.

## Methods

Four shore pine trees (approximately 20 cm in diameter and 9 m long) were felled on May 4, 2001. The trees were located in Alaska: Juneau Borough, Douglas Island, 1.1 km W Eaglecrest Ski Area, 58°31'N, 134°37'W. To insure that the trees would be colonized by *I. pini*, two trees were baited with the pheromone for the eastern population of *I. pini* [racemic ipsdienol (equal mixture of the (+)- and (–)-enantiomers) and lanierone] and two trees were baited with the pheromone for the western population of *I. pini* [>97% -(–)-ipsdienol]. All materials were released from bubble cap release devices at sub-milligram rates per day (Phero Tech Inc.).

On June 15, 2001 infested logs were mailed to Seybold for the rearing and identification of bark beetles. Upon receipt in Minnesota, a quick examination of the logs showed that beetles at similar densities colonized both sets of logs. The logs were placed into large wooden rearing boxes (Browne, 1972); insects emerged between July and September 2001. Specimens of *I. pini* from these logs were frozen (-80°C) for later use in analyses of mitochondrial and nuclear DNA (in cooperation with A. Cognato, Texas A&M Univ.) and cuticular hydrocarbons (Seybold). Specimens of female *I. pini* were also used in a scanning electron microscopic study of the pars stridens (sound-producing organ on the head). The morphology and interstrial distance of this structure for the population from southeastern Alaska matched those of three other North American populations (Santiago-Blay et al., unpublished).

Live *I. pini* that emerged from the cut logs were separated by sex and reintroduced individually into smaller green logs of *P. c. contorta*. At least one hundred beetles were placed into each log. These artificially infested logs were immediately placed into glass carboys and volatiles were trapped from above the logs for about 8 days (beginning on July 31, 2001). Volatiles were trapped on a resin called Porapak Q (methods summarized in Seybold et al., 1995). This procedure resulted in four samples: 1) Male *I. pini* that had responded to the eastern bait; 2) Female *I. pini* that had responded to the eastern bait; 3) Male *I. pini* that had responded to the western bait; and 4) Female *I. pini* that had responded to the western bait. These Porapak Q samples were placed in a freezer (-20°C) until they were extracted in pentane with an internal standard (4-decanone) in April 2002. Analyses of the samples by gas chromatography with flame ionization detection (GC-FID) revealed surprisingly small amounts of ipsdienol in the male samples, making it difficult to measure the enantiomeric composition (stereochemistry) of the ipsdienol. The column used for the analysis was

HP-Wax (Agilent). The same samples were further analyzed by gas chromatography-mass spectrometry (GC-MS) using the same column and separation conditions.

## Results

The shore pine that had been felled was checked on May 14 and 21, 2001, 10 and 17 days after baiting, and no attacks were found. These felled trees were again checked on May 30, 2001 and those trees baited with the eastern lure baited were fully attacked. Only one attack was found on the felled trees baited with the western lure, suggesting that Alaska populations of *I. pini* may prefer the racemic blend of ipsdienol. However, after May 30, the felled trees baited with the western lure were colonized at a density that matched the felled trees baited with the eastern lure.

Only two species of bark beetles emerged from the logs cut from the felled trees: *I. pini* and a root feeding bark beetle, *Hylurgops rugipennis rugipennis* (Mann.). The latter species likely attacked the portions of the felled trees that were in contact with the ground. Specimens of both species were curated and sent to an internationally recognized expert in bark beetle taxonomy (Dr. Donald Bright, Ottawa, Canada) and voucher specimens of each species were deposited in the USDA Forest Service PNW collection in Juneau, in the California Academy of Sciences Insect Collection in San Francisco, and in the University of Minnesota Insect Collection in St. Paul. The lengths of the *I. pini* from Alaska were measured and they all fell within the published range for *I. pini* (3.3 to 4.5 mm). This collection from Alaska was documented in a short review of the distribution and host range of *I. pini* (Gandhi and Seybold, 2002).

Male *I. pini* volatile samples contained fairly large amounts of amitinol (a derivative of ipsdienol), small amounts of ipsdienol and trans-verbenol, and traces of the compounds E-myrcenol (another compound related to ipsdienol) and myrtenol (a ß-pinene derivative). Female volatile samples also contained small amounts of trans-verbenol and traces of myrtenol, but generally lacked amitinol, ipsdienol, and E-myrcenol. There was a slight trace of ipsdienol in the volatile sample from the females that responded to the western bait, but this could have resulted from one or several males mistakenly placed into the artificially infested logs. Otherwise, the eastern and western volatile samples were quite similar across both sexes.

## Summary

In summary, volatile samples from male *I. pini* from Alaska are unusual for western and eastern populations of *I. pini* in that they appear to contain large quantities of amitinol. This could result from actual production of the compound by the beetles or through acid rearrangement of ipsdienol during collection, extraction, storage, or analysis of the samples (Francke et al., 1980). The quantity of ipsdienol in these samples was lower than it usually is in samples from *I. pini* in other parts of western North America. This has prevented an easy determination of the enantiomeric composition of the ipsdienol. The presence of trans-verbenol in the extracts is not unusual for *I. pini*, but may have implications for this particular population. The behavioral activity of these compounds for Alaskan populations of *I. pini* could be tested in the future using GC-EAD. Further, another collection of *I. pini* from this location in Alaska could be made using only the eastern or the western bait to attract more insects to produce an extract containing more ipsdienol. If indeed the large quantities of amitinol were not an artifact of sample processing or storage, its importance in the behavior of *I. pini* would require future laboratory (e.g. GC-EAD) or field studies. The extracts from the 2001 samples are still at Seybold's lab and there is the potential to fractionate the extracts by HPLC to isolate the ipsdienol for stereochemical analysis.

## Acknowledgements

We thank the Juneau Ranger District, especially David Carr, for providing the shore pine for this study.

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# Appendix A

## Pine Engraver Beetle
## *Ips pini* (Say)

**Hosts**: *Pinus* spp.; rarely *Picea* spp.

**Distribution**: Eastern to western coasts of North America and from Alaska to northern Mexico.

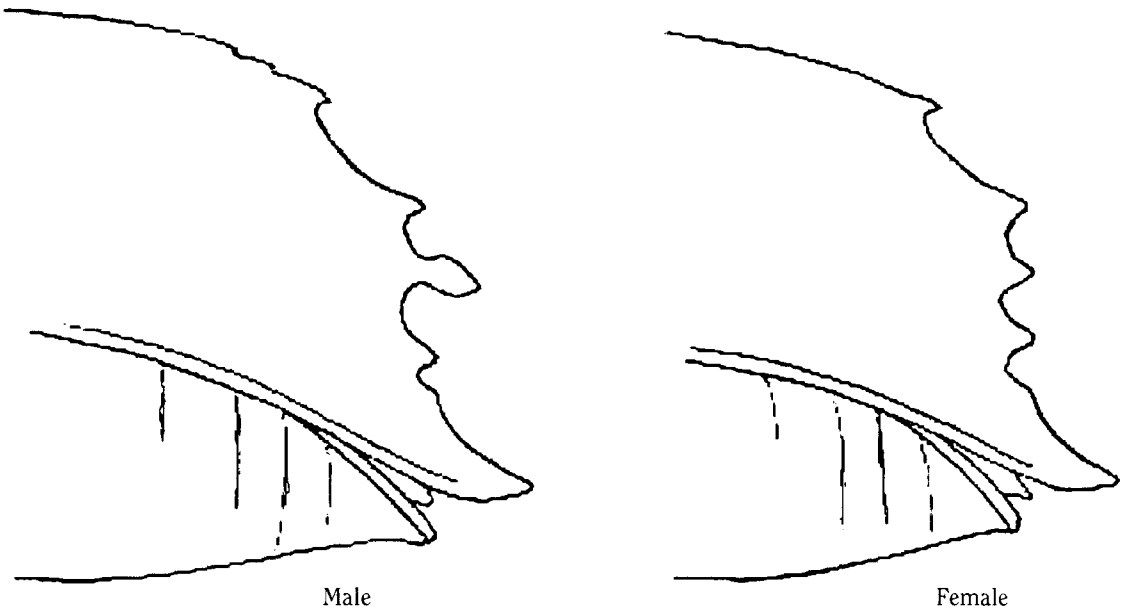
**Description**: Adults are small (3.3–4.3 mm long) dark reddish brown to almost black. The head is not visible when the insect is viewed from above. A distinguishing feature of all *Ips* is a pronounced declivity (the sloping, sometime concave, rear end of the elytra [wing covers]) with four teeth on each elytra (Figure 1). The third tooth is larger and capitate in the male, but equal to the second tooth in the female.

Larvae are segmented, soft-bodied, whitish, elongate, cylindrical, legless grubs. Head color is tan, and the thoracic segment is enlarged. Eggs are fragile, globular to ovoid in shape and translucently white. They are located in niches or grooves along the sides of egg galleries.

**Biology**: The life cycle of *I. pini* is probably one to two generations per year in Alaska. The winter is spent almost exclusively in the adult stage, either under the bark or in forest litter. The first evidence of *Ips* attack is fine, yellow-red boring dust in bark crevices or more commonly encountered on the topside of down host material. Adults disperse in new host material in May–June to infest slash (live pines are not susceptible then). Male *Ips* begins tree attacks, and males and females are soon attracted via pheromones. *Ips* aggregate on selected hosts in response to male produced pheromones in the initial galleries. Two or more aggregating pheromones, along with host volatiles direct aggregation. After boring into the bark, males construct an enlarged chamber in the inner bark adjacent to the entrance hole. Adults construct egg galleries, which radiate from the central chamber. These egg galleries are kept clear of frass and boring dust. Larvae bore outwards from the egg galleries.

Preventing these beetles from becoming too numerous through timely slash disposal and thinning of dense immature stands will do more to prevent damage than the application of control measures after damage has occurred.

***Figure 1. Declivity teeth.***



Male Female

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